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Prevalence and distribution of antimicrobial resistance in clinical mastitis in Danish dairy cows

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Aim of study

The antimicrobial agents used to treat clinical mastitis in Danish dairy cows are primarily penicillins, which made up about 81% of the total kg active compounds used for intramammary treatment in 2016 (DANMAP 2016). The present study examines the resistance patterns of 299 bacterial isolates from Danish dairy cows with clinical mastitis. The aim of the study was to provide resistance data to serve as a guideline for prudent use of antimicrobial agents in mastitis treatment. Additionally, the study serves as a baseline for future comparison of resistance patterns and distributions.

Materials and Methods

A total of 299 samples of either bacterial cultures or milk were obtained from clinical mastitis. All samples were obtained in Danish veterinary practices in 2016 and submitted for further laboratory investigation to the National Veterinary Institute, DTU. The bacterial isolates and milk samples were (sub)cultured onto blood agar plates. All plates were incubated at 37°C and read after 24h and, when relevant, 48h. Dominant colonies were identified by matrix-associated laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF MS) and kept as freeze-isolates until further examination. For each isolate identified by MALDI-TOF a minimum inhibitory concentrations (MIC)-determination was carried out by broth microdilution using a semi-automatic system (SensiTitre, TREK Diagnostic Systems, UK). The occurrence of resistance was evaluated using CLSI clinical breakpoints or in-house breakpoint values. Resistance percentages were calculated for isolates that displayed MIC-values above the breakpoint for resistance.

Main results and conclusions

From the 299 samples in total, MICs were determined in *Escherichia coli* (n=62), *Klebsiella pneumoniae* (n=18), *Staphylococcus aureus* (n=63), Coagulase Negative Staphylococci (n=49), *Streptococcus agalactiae* (n=13), *Streptococcus dysgalactiae* (n=33) and *Streptococcus uberis* (n=61). Here we report the findings of ≥ 10% resistance prevalence.

In the *E. coli* isolates, resistance prevalence was 17.7% to sulphamethoxazole, 16.1% to trimethoprim, 12.9% to streptomycin, 11.3% to tetracycline and 11.3% to ampicillin. Our findings in *E. coli* resemble but were slightly higher than resistance patterns reported from Sweden or Canada (Bengtsson et al. 2009, Saini et al. 2012).

In the *K. pneumoniae* isolates 83.3% displayed resistance to ampicillin, while all isolates were susceptible to the rest of the panel of antimicrobial agents tested, except for streptomycin. Our findings in Klebsiella also resemble resistance patterns reported from Sweden, where 97.6% of Klebsiella isolates from mastitis were resistant to ampicillin (Bengtsson et al. 2009).

In the *S. aureus* isolates, resistance prevalence was 52.4% to spectinomycin, 28.6% to sulphamethoxaxole and, 17.5% to penicillin. In addition, one *S. aureus* isolate was identified as LA-MRSA.

All streptococci were sensitive to penicillin. All the *S. agalactiae* isolates (100%) were streptomycin resistant and the majority (76.9%) were resistant to tetracycline as well. A whole genome sequencing study of the *S. agalactiae* isolates is now in progress to reveal the genes causing resistance in *S. agalactiae*. In the *S. dysgalactiae* isolates, resistance was 12.1% to streptomycin. In the *S. uberis* isolates, resistance prevalence was 100% to sulphamethoxazole, 98.4% to streptomycin, 21.3% to tetracycline, and 18.0% to cefoxitin.

Among the 49 CNS isolates, the most prevalent species were *S. simulans* (n = 13), *S. chromogenes* (n=11) and *S. epidermidis* (n=9). In the CNS isolates, resistance prevalence was 22% to penicillin, 20% to sulphamethoxazole and 10% to each of the antimicrobials spectinomycin, tetracycline and tiamulin, respectively. One *S. chromogenes*-isolate was identified as LA-MRSA. A study on CNS isolates from milk and teat skin of Danish dairy cows, showed a different distribution of dominant CNS-species (Mahmmod et al. 2017). This suggests that udder-pathogenicity varies between CNS-species since their distribution apparently varies between clinical mastitis and subclinical/non-mastitis milk.

In conclusion, overall occurrence of resistance to most antimicrobial agents tested in this study was low. However, since tetracycline, spectinomycin, sulphamethoxazole and streptomycin is not used/used scarcely for intramammary treatment of mastitis in Denmark (DANMAP 2016) the high resistance prevalence in certain bacteria to these drugs is not related to consumption. Accordingly, some of the isolates needs to be further investigated to explain their resistance prevalence.

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